SUPPLEMENTARY MATERIALS

Serum microRNAs are early indicators of survival after radiation-induced hematopoietic injury

Sanket S. Acharya[1], Wojciech Fendler[2], Patryk Moskwa[3], Jacqueline Watson[1], Abigail Hamilton[1], Yunfeng Pan[1], Payel Bhanja[4], Emily Gaudiano[1], Subhrajit Saha[4], Chandan Guha[4,5], Kalindi Parmar[1] and Dipanjan Chowdhury[1†]

MATERIALS AND METHODS

Study Design

This study was designed to study whether serum miRNAs can predict radiation-induced hematopoieitc damage early after radiation exposure. Differential expression of miRNAs in response to radiation was investigated in two model systems, strongly suggesting the applicability of miRNAs as radiation-specific markers of latent hematopoietic injury. Animals were also treated with radioprotective and radiomitigating agents to correlate levels of specific mIRNAs with animal survival at 30 days post radiation. All animal procedures performed were approved by the Institutional Animal Care and Use Committee (IACUC) at Dana-Farber Cancer Institute. Animals were maintained in the animal facility and given *ad libitum* access to food and water. Body condition scores (BCS) as described in (*43*) was used to standardize endpoints. A BCS of 3 was regarded as the endpoint for all irradiated animals irrespective of treatment group, at which point animals were considered moribund and euthanized. Mouse serum samples used for miRNA profiling were randomized before analysis and experiments were generally repeated three times.

Power Analysis: Power analysis was performed using the Hierarchical Clustering Explorer 3.5 tool (*44*). The number of samples was estimated to be sufficient to provide statistical power of at least 80% needed to obtain a p value of less than 0.01 for differentially expressed miRNAs with a fold change 0 >1.5 or <0.67 in between group comparisons. The p value threshold was lowered from 0.05 to account for multi-group post-hoc testing. A sample size of 10 per group was thus calculated to allow us to confirm statistically significant differences for the top 95 differentially expressed

miRNAs with the predetermined effect sizes. *P* levels lower than 0.05 were considered as statistically significant.

Mice and Total Body Irradiation

C57BL/6J male mice (10 week old) were obtained from Jackson Labs (Bar Harbor, Maine) and acclimated in the Animal Research Facility (ARF) at the Dana-Farber Cancer Institute before irradiation at the age of 12-13 weeks. All procedures performed were approved by IACUC at DFCI. Animals were exposed to TBI in an irradiation pie cage (Braintree Scientific) at various doses. Irradiation was performed using a ^{137}Cs source at a dose rate of 110 cGy/min using a 137 Cs source (Gamma Cell® 40 Exactor, Best Theratronics, Ottawa, Canada). Instrument calibration is performed according to vendor instructions in accordance with the DFCI Office of Radiation Safety.

Bone marrow Harvest and Flow Cytometry

Bone marrow was harvested as per protocols described in (*45*). Briefly, animals were dissected to isolate femurs and tibia from mouse hind limb. Extracted bones were flushed with a 23 gauge needle using Hank's Balanced Salt Solution (HBSS, Gibco) supplemented with 2% Fetal Bovine Serum (FBS) and 1% 10mM HEPES (Gibco) to obtain bone marrow. Cells were then passed through an 18 gauge needle to obtain a single cell suspension. BM-MNC count was determined by counting cells using 3% Acetic Acid with Methylene Blue Solution (Stem Cell Technologies). For LKS (lineage, cKit, Sca1) staining to visualize HPCs and HSCs, whole bone marrow was stained with biotinylated anti-lineage cocktail (anti-Mac1, Gr-1, CD3e, B220, and Ter119), APC-

conjugated anti-cKit (clone 2B8), and PECy7-conjugated anti-Sca1 (clone D7) antibodies. Following primary antibody staining, cells were washed and incubated in PE-conjugated streptavidin secondary antibody to visualize lineage positive cells. All primary and secondary antibodies were obtained from BD Biosciences. Samples were acquired using an LSR Fortessa instrument (BD) and data was analyzed using FlowJo software (TreeStar).

Complete Blood Counts (CBCs)

Blood collection for CBCs (100 μl) was performed by retro-orbital bleeding after anesthesia in EDTA-coated tubes (BD Biosciences). CBCs were recorded with a Hemavet 950 FS hematology analyzer (Drew Scientific).

Colony Assays

To assess colony-forming ability, whole bone marrow isolated after flushing mouse femurs and tibiae was plated in 12-well plates at a density of 20,000 and 100,000 cells/well in mouse or human methylcellulose medium (Stem Cell Technologies). Cells from all samples were plated in triplicates and incubated at 37^0C in 5% CO_2 for 7 days at which time hematopoieitic colonies formed (colony-forming units in culture, CFU-Cs) were scored.

HSC and Bone Marrow Transplantation

Short-term and long-term repopulating ability was assessed by transplantation of either sorted HSCs or unfractionated whole bone marrow from donor mice (C56BL/6J CD45.2 congenic) into lethally irradiated (10 Gy) recipients (B6.SJL-Ptprc^a Pep3^b/BoyJ CD45.1

congenic) as described in (*45*). Donor mice were exposed to 2 and 6.5 Gy TBI and allowed to recover for 3 months, at which time animals were sacrificed, bone marrow was isolated by flushing, and HSCs were sorted on a FACSAria (BD Biosciences). For transplants involving sorted HSCs, a total of 2000 LKS+ cells from CD45.2 donor mice were mixed with 250,000 support bone marrow cells (CD45.1 positive) and injected IV per lethally irradiated CD45.1 recipient. For transplants involving unfractionated bone marrow, a total of 500,000 whole bone marrow cells from CD45.2 donor mice were mixed with 250,000 support bone marrow cells (CD45.1 positive) and injected IV per lethally irradiated CD45.1 recipient. Five mice were transplanted per TBI dose group for HSC transplants while four mice were transplanted per TBI dose group for whole bone marrow transplant. Peripheral blood samples collected at 1 and 4 months post transplant were used to assess short-term and long-term repopulation, respectively. Donor-cell chimerism in recipients was assessed by staining peripheral blood with FITC-conjugated anti-CD45.2 (clone 104) and PE-conjugated anti-CD45.1 (clone A20) antibodies. To measure the extent of multi-lineage reconstitution, the percentage of donor-derived B cells, T cells, and myeloid cells was calculated by co-staining with PE-conjugated anti-B220 (clone RA3-6B2), PE-anti-CD3e (clone 145-2C11), and PE- anti-Mac1/anti-Gr1 (clones M1/70 and RB6-8C5), respectively. All antibodies were obtained from BD Biosciences. Stained samples were acquired on an LSR Fortessa instrument (BD) and analyzed using FlowJo software (TreeStar).

Serum Preparation

Peripheral blood was collected by retro-orbital bleeding after anesthesia. Upto 200 µl of blood was collected in DNAse/RNAse- free eppendorf tubes and incubated at room

temperature (RT) for 2h to allow clotting. Blood samples were then spun in an Eppendorf 5415C centrifuge at 14000 rpm (15996g) for 5 min at RT. The supernatant was collected and re-spun at the above conditions to remove any remaining cellular contamination. The resulting supernatant (serum) was stored in aliquots at -80 ^{0}C.

Murine miRNA Profiling

miRCURY LNA^{TM} Universal RT miRNA PCR Rodent Panel I&II containing 742 assays were used to profile miRNAs differentially expressed in mouse serum from animals exposed to 0 Gy (control), 2 Gy, 6.5 Gy, and 8 Gy doses of TBI (Exiqon). Ten mice were profiled per group for a total of 40 samples. On average 339 miRNAs were detected per sample, at least 170 assays was detected in all samples, and 68 of these miRNAs were differentially expressed with a p value below 0.05 (fig. S5A and table S2). Please refer to table S2 for raw expression values and ranking of all miRNAs significantly altered during profiling. Data quality for samples across different groups was determined by comparing number of detected miRNAs with overall Cp values and was found to be very similar. Normalization of data was performed using the global mean of 170 miRNAs most-commonly expressed in all samples. A set of RNA and DNA spike-in controls and hemolysis controls were also incorporated to ascertain the technical performance of each sample (fig. S5B and S5C). Spike-in controls were used throughout the study for profiling and validation. RNA spike-in controls were used to test the efficiency of the cDNA synthesis reaction while DNA spike-in control tested the efficiency of qPCR amplification. In order to negate the possibility of hemolysis, ΔCp for miR-451 (expressed in red blood cells) and miR-23a-3p (relatively stable in serum) was computed

for each sample as previously reported (*46*). ΔCp values lower than 7 suggest minimal levels of red blood cell contamination.

RNA Extraction and cDNA Synthesis

Total RNA was isolated from serum samples by using the miRCURY^{TM} RNA Isolation Kit – Biofluids from 50 µl mouse serum as per manufacturer's manual. Total RNA was eluted in 50 µl of RNAse-free H_2O and stored at -80^{0}C long-term. Since only small RNA can be isolated, RNA concentration measurement by Nanodrop or spectrophotometry is not reliable. Hence, as per manufacturer's recommendations, input volumes for serum RNA were optimized for the cDNA synthesis reaction. cDNA was synthesized in 10 µl reactions using the Universal cDNA Synthesis Kit II and was diluted 50-fold in RNAase/DNAse-free H_2O for use in quantitative PCR. Reagents for RNA extraction and cDNA synthesis obtained from Exiqon.

Quantitative PCR

Diluted cDNA was subjected to quantitative PCR analysis in Pick-N-Mix plates designed in a 96-well format. SYBR® Green qPCR MasterMix was mixed 1:1 with diluted cDNA and added to specific wells in pre-designed Pick-N-Mix plates containing dried-down LNA primers specific for selected miRNAs (see table S3 for a list of miRNA target sequences). The Pick-N-Mix plates also contained number of controls including miR-101a and miR-19b (normalization controls), UniSp6 (proprietary RNA spike-in control) and UniSp3 (proprietary DNA spike-in control). Built-in interplate calibtrator (IPC)

reactions were used to control for inter-plate variability. Pick-N-Mix qPCR plates were run on an Applied Biosystems 7500 FAST Real-Time PCR System. Data was generally normalized using miR-101a. However, normalization using miR-19b produced similar results. miR-451 and miR-23a were used to assess extent of hemolysis. All reagents used for quantitative PCR were obtained from Exiqon.

Radioprotection with Amifostine

Amifostine was given to both C57BL/6J and huCD34+ NSG 'humanized' mice intraperitoneally (IP) at 200 mg/kg body weight 1 hour prior to TBI.

Bone marrow adherent stromal cell (BMASC) culture and transplantation

All studies were performed under the guidelines and protocols of the Institutional Animal Care and Use Committee (IACUC) of the Albert Einstein College of Medicine. The animal use protocol for this study was reviewed and approved by IACUC at Albert Einstein. Five- to 6-weeks-old male C57Bl/6 (NCI-Fort Dietrich, MD) mice were maintained *ad libitum*. TBI (10.4 Gy) was performed on anesthetized animals (intraperitoneal ketamine and xylazine 7:1 mg/ml for 100µl/mouse) using a Shephard^{137}Cs -ray irradiator at a dose rate of 236 cGy/min following biosafety guidelines of the Albert Einstein College of Medicine. Donor bone marrow cells were harvested using sterile techniques from the long bones of C57Bl/6 mice and cultured in mesenchymal stem cell (MSC) basal medium (Cambrex-Lonza, Walkersville, MD) supplemented with 10% heat inactivated FBS, 1% Glutamine, and 1%

Penicillin/Streptomycin for 4 days. This was followed by collection of adherent cells, termed bone marrow derived adherent stromal cells (BMASCs). BMASCs (2×10^6 cells/mouse) were injected intravenously via tail vein into C57BL/6J recipients at 24 and 72 hours after irradiation.

HuCD34+ NOD *scid* gamma chain deficient (NSG) mice

HuCD34+ "humanized" NSG mice were obtained from Jackson Labs, Bar Harbor, ME and housed in a BL2 facility at DFCI. All procedures performed were approved by the Institutional Animal Care and Use Committee (IACUC) at DFCI. During the generation of these mice at Jackson Labs, 3 week old female NSG mice were irradiated at 1.4 Gy to deplete their bone marrow and injected with CD34+ human HSC. At 12 weeks after transplant, each mouse was tested by FACS for engraftment of human CD45+ and murine CD45+ cells. Animals were obtained at DFCI approximately 10 weeks after engraftment confirmation at Jackson Labs. Peripheral blood and bone marrow from animals in the untreated Control arm was reconfirmed for the presence of human CD45+ cells at DFCI with anti-human CD45 FITC (clone 2D1 from BD). Refer to Fig.5D, Table S4, and http://jaxservices.jax.org/invivo/humanized-cd34-mice.html.

Statistical Analysis

MicroRNA Profiling: Normalization of miRNA serum levels was performed using 170 commonly expressed miRNA. Analysis of variance (ANOVA) was used to determine which miRNAs differed significantly between groups. To adjust for multiple comparisons testing the Benjamini-Hochberg correction was applied. A threshold of

$p<0.05$ in ANOVA was selected as the level of statistical significance. Tukey's test was used to determine between-group significance in post-hoc comparisons. MiRNAs with p values <0.05 in ANOVA were used in hierarchical-clustering analysis to visualize expression patterns. Differentially expressed miRNAs were tested in pairwise comparisons with a Benjamini-Hochberg adjusted Student's t-test to determine between-group differences.

Validation with real-time qPCR: One-way ANOVA was used to confirm global significance. Dunnett's post-hoc testing procedure was used to compare miRNA levels in the 8Gy + Saline group against the other three experimental groups. Univariate comparisons were performed using the student's t-test or the student's t-test for paired samples. Pearson's correlation coefficient was used for correlation testing. Survival analysis was performed using the log-rank (Mantel-Cox) test.

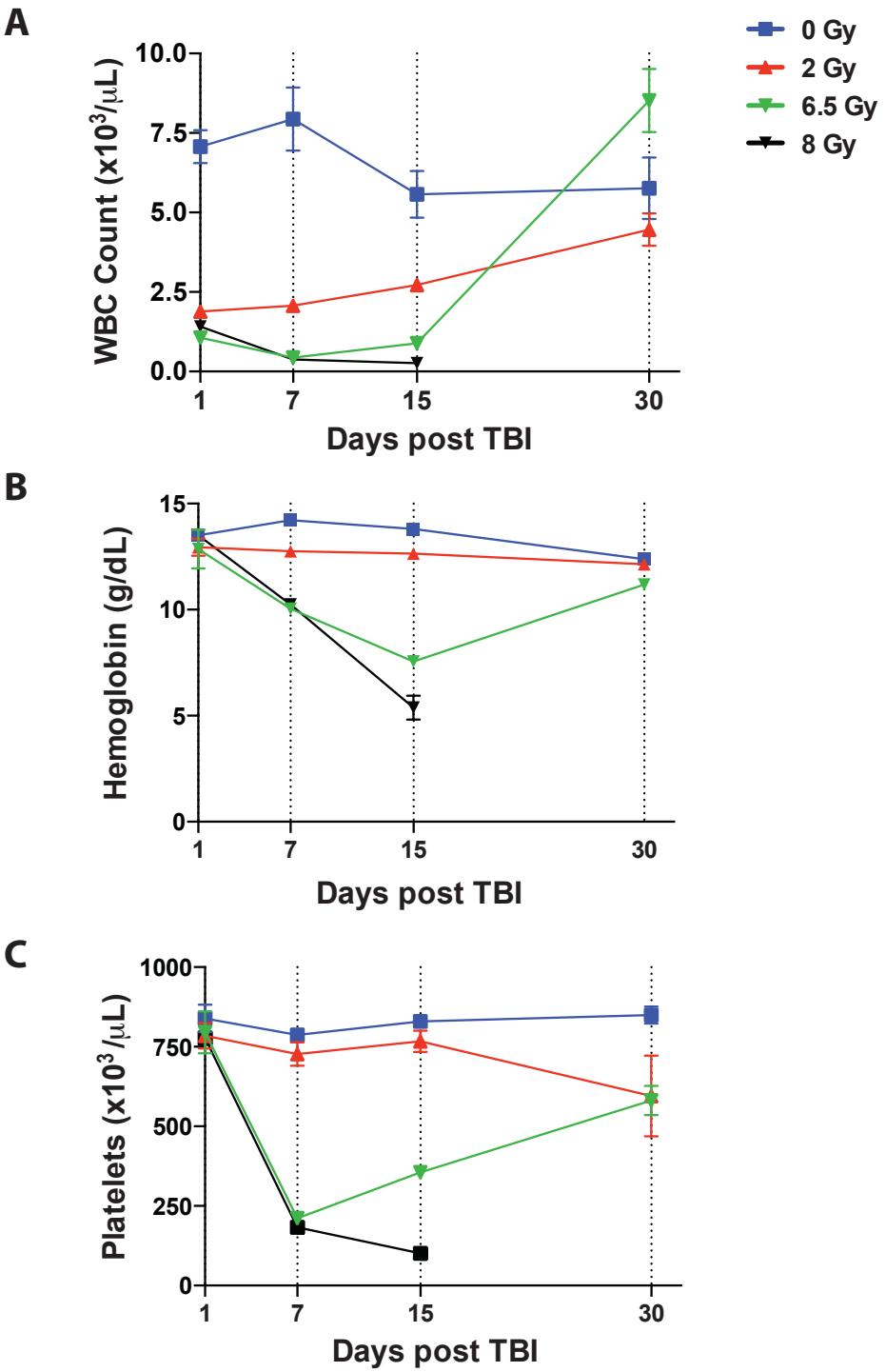


Fig. S1: Analysis of peripheral blood CBC parameters following TBI-induced hematopoietic damage. Total WBC count **(A)**, hemoglobin **(B),** and platelets **(C)** at 24 hr (day 1), 7, 15, and 30 are shown for mice irradiated with the indicated doses (n =5

mice/group/timepoint). The same mice were used for bone marrow analysis reported in Fig. 1. Control animals maintained stable levels of all parameters. WBC count in animals exposed to all TBI doses dropped sharply 24 hr post TBI ($p< 0.0001$) and remained low until day 15 ($p< 0.001$). By day 30, the 8 Gy animals were not viable but the 2 and 6.5 Gy cohorts showed near complete recovery in their WBC levels. Hemoglobin levels in all irradiated cohorts were unaffected at 24 hr. At day 7 and day 15, all TBI cohorts showed decreased hemoglobin levels and the 6.5 and 8 Gy cohorts were anemic ($p< 0.0001$). By day 30, hemoglobin levels of the 2 and 6.5 Gy cohorts were close to normal while the 8 Gy cohort was not viable. A similar trend was seen for platelet depletion and recovery. Statistical significance assessed by one-way ANOVA followed by Dunnett's test for multiple comparisons (also see table S1).

Supplementary Fig. 2

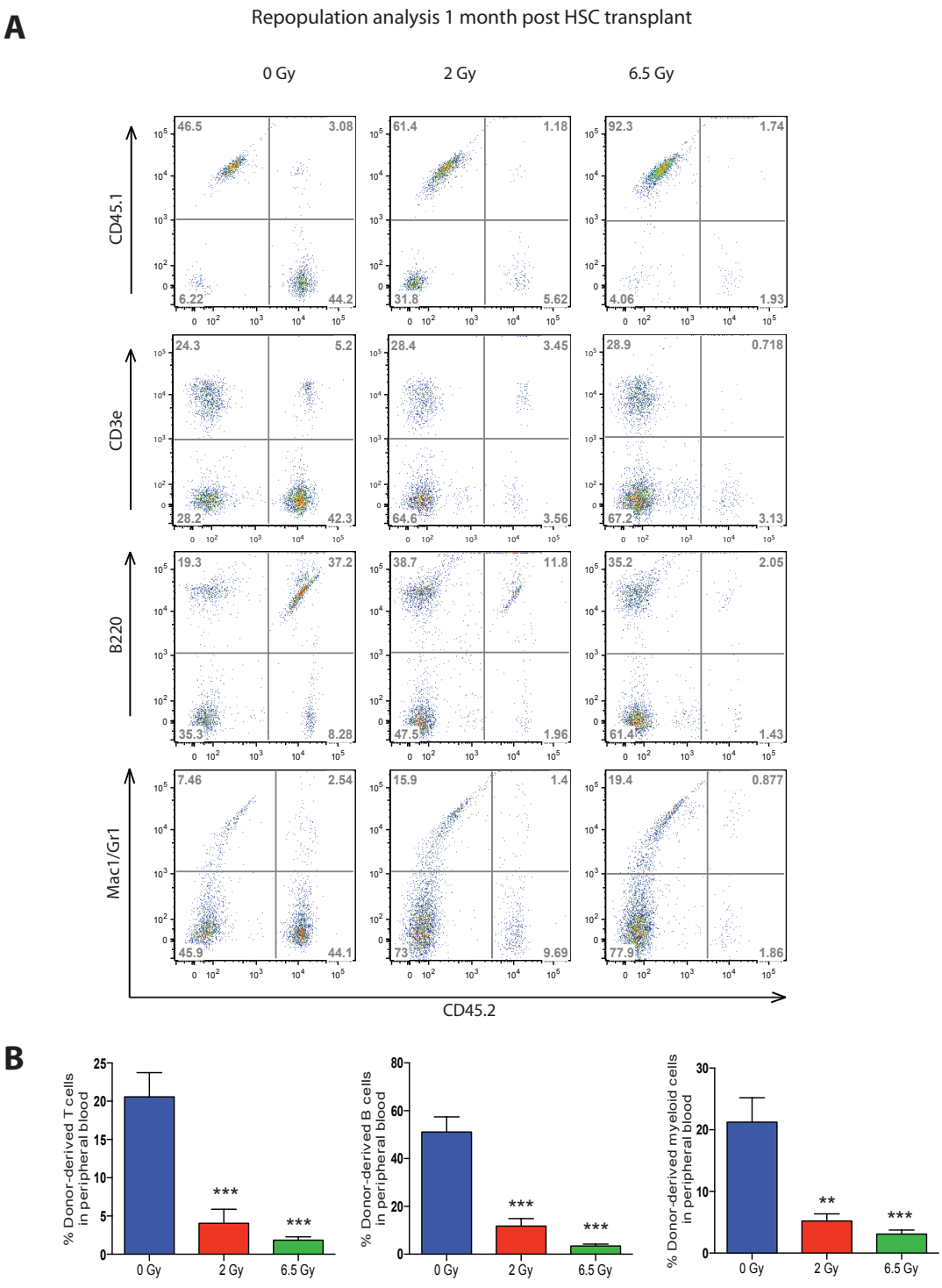


Fig.S2: Repopulation analysis at 1 month post HSC transplant. C56BL/6J CD45.2 donor mice were left un-irradiated (control 0 Gy) or exposed to TBI doses of 2 and 6.5 Gy and allowed to recover for 3 months. At 3 months, mice were sacrificed and bone marrow resident HSCs (LKS+ population) were sorted from (n=5/ group). Following sorting, HSCs from each group were pooled and then mixed with supporting bone marrow cells obtained from recipient CD45.1 mice. A total of 2000 sorted HSCs were injected together with 250,000 supporting whole bone marrow cells in recipient animals (n =5/ transplant group). **(A)** Representative FACS plots of donor-cell chimerism measured at 1 month post transplant. Peripheral blood from recipient animals was harvested and stained with antibodies against CD45.1 (recipient marker), CD45.2 (donor marker), CD3e (T cells), B220 (B cells), and Mac1/Gr1 (myeloid cells). **(B)** Graphical representation of multilineage reconstitution in recipients transplanted with HSCs. Percent donor-derived T, B, and myeloid cells at 1 month post-transplant are shown. Total leukocyte chimerism at 1 month is shown in Fig 2D.

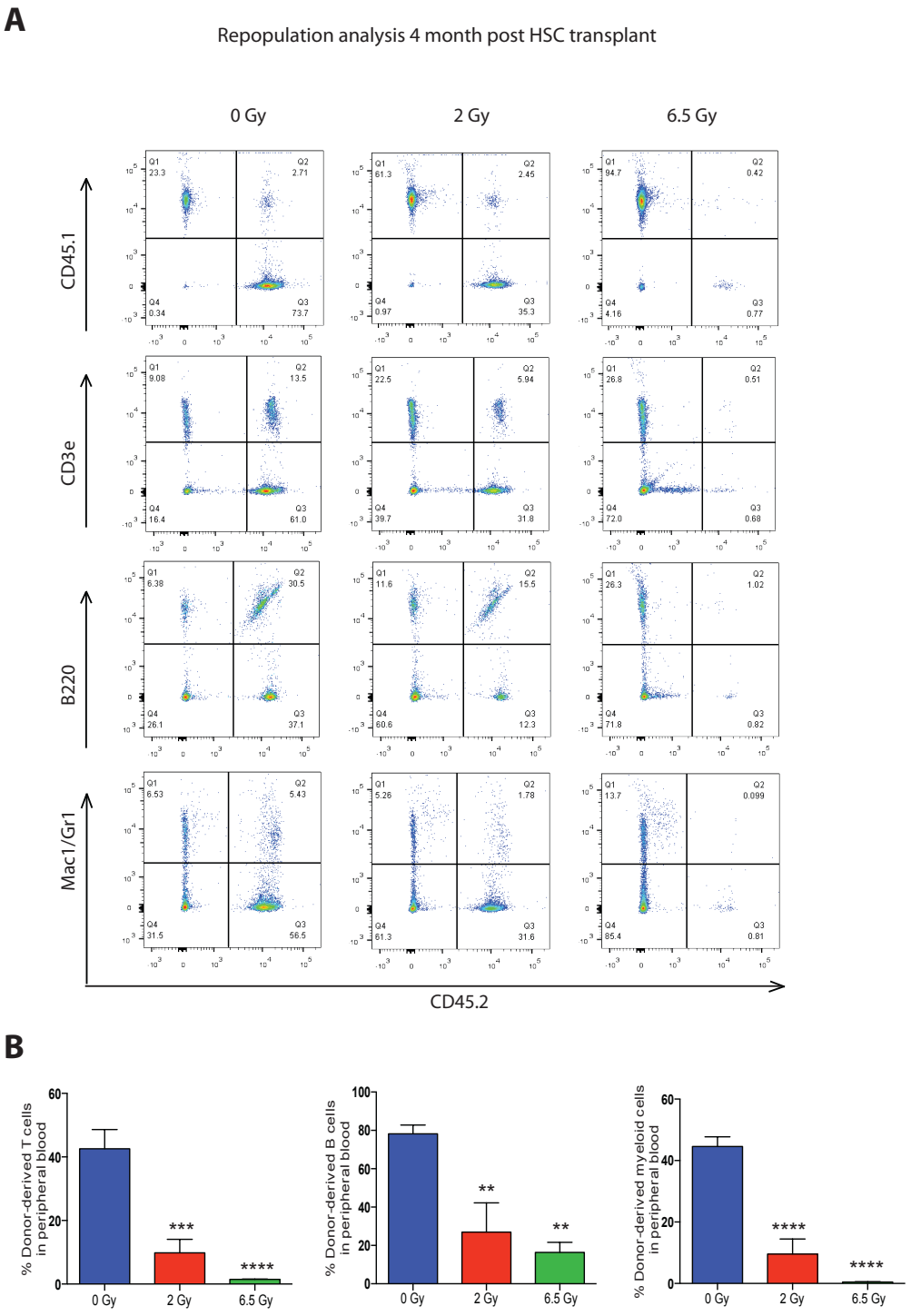


Fig. S3: Repopulation analysis at 4 months post HSC transplant. Experiment was performed as described in fig. S2. **(A)** Represenative FACS plots of donor-cell chimerism in peripheral blood measured at 4 months post-transplant are shown. Peripheral blood

from recipient animals was harvested and stained with antibodies against CD45.1 (recipient), CD45.2 (donor), CD3e (T cells), B220 (B cells), and Mac1/Gr1 (myeloid cells). **(B)** Graphical representation of multilineage reconstitution in recipients transplanted with HSCs. Percent donor-derived T, B, and myeloid cells at 4 months are shown. Total leukocyte chimerism at 4 months is shown in Fig 2E.

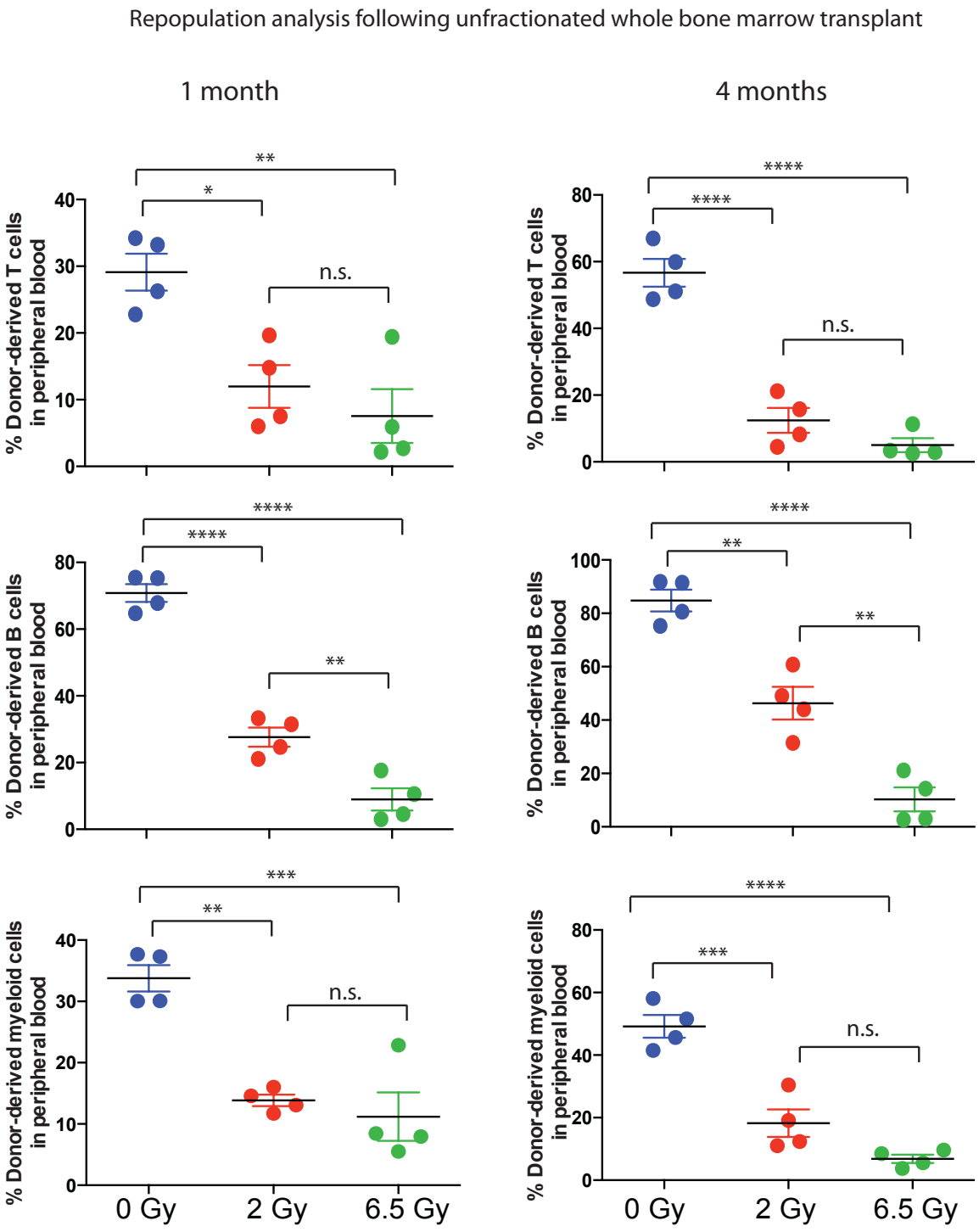
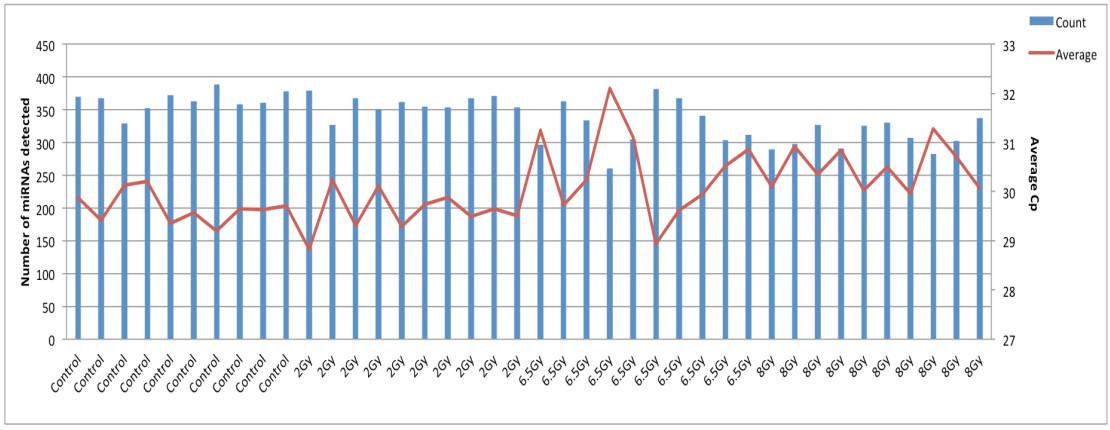


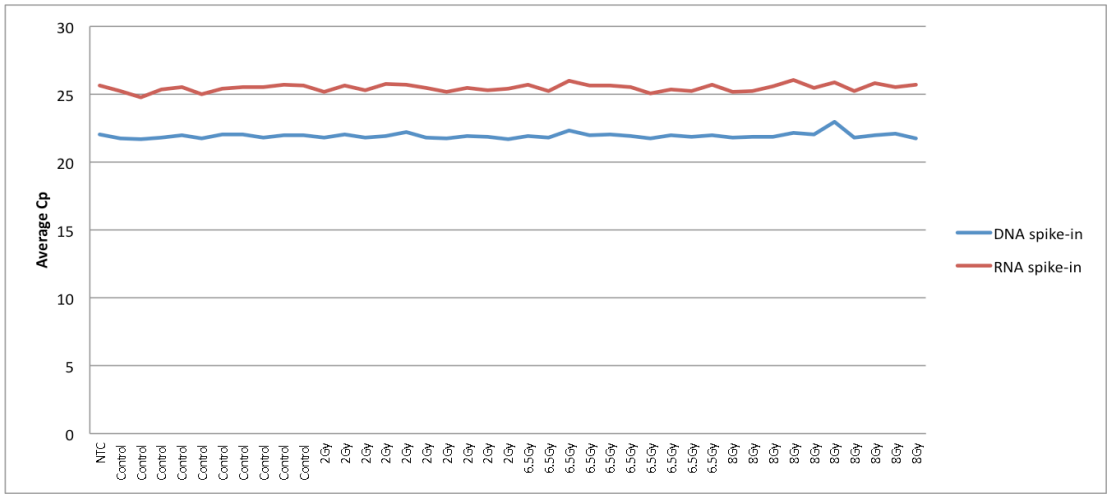
Fig.S4: Repopulation analysis following unfractionated whole bone marrow transplant. C57BL/6J mice (n =5/ group) were left unirradiated (0 Gy) or exposed to 2 or 6.5 Gy TBI and allowed to recover for 3 months. At this time, WBM from these mice

was isolated and mixed with supporting CD45.1 bone marrow. A total of 500,000 CD45.2 WBM cells and 250,000 CD45.1 support cells were injected in lethally irradiated CD45.1 recipients (n=4 / group). Peripheral blood from recipient animals was harvested and stained with antibodies against CD45.1 (recipient), CD45.2 (donor), CD3e (T cells), B220 (B cells), and Mac1/Gr1 (myeloid cells). Multilineage (T, B, and myeloid cell) reconstuitution at 1 month and 4-months post WBM transplant are presented. Results of total leukocyte chimerism at 1 and 4 months are presented in Fig.2D and 2E, respectively. Asterisks identify statistically significant comparisons. One-way ANOVA followed by Tukey's test for multiple comparisons was used to assess statistical significance. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$; n.s., not significant.

A


Count
Average
Number of miRNAs detected
Average Cp
450
400
350
300
250
200
150
100
50
0
33
32
31
30
29
28
27
Control
2Gy
6.5Gy
8Gy

B


Average Cp
30
25
20
15
10
5
0
NTC
Control
2Gy
6.5Gy
8Gy
DNA spike-in
RNA spike-in

C

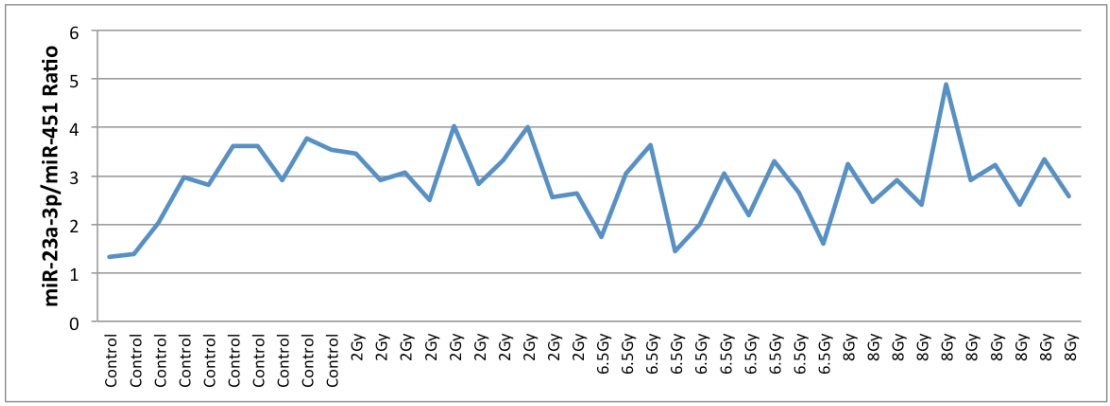

miR-23a-3p/miR-451 Ratio
6
5
4
3
2
1
0
Control
2Gy
6.5Gy
8Gy

Fig.S5: Serum miRNA profiling. (A) C57BL/6J mice (n = 10/ group) were exposed to 0 Gy, 2 Gy, 6.5 Gy, and 8 Gy TBI. Serum collected from these animals 24 hr after TBI was subjected to circulating miRNA profiling using the miRCURY LNA Universal RT miRNA PCR Rodent Panel I and II (see Materials and Methods). Number of miRNAs detected per sample (count) and the average amplification threshold (Cp) value for miRNAs in each sample are presented. **(B)** Built-in RNA and DNA spike-in controls were used to test technical performance of samples used for profiling. Graph shows the average Cp value for detecting proprietary spike-in RNA (UniSp6) and spike-in DNA (UniSp3). Technical performance of all samples was found to be very similar. **(C)** Hemolysis controls were used in the profiling and subsequent validation to rule-out red blood cell contamination of circulating miRNAs. A combination of miR-451 (expressed in red blood cells) and miR-23a-3p (relatively stable in serum) was used to estimate likelihood of hemolysis (see Materials and Methods).

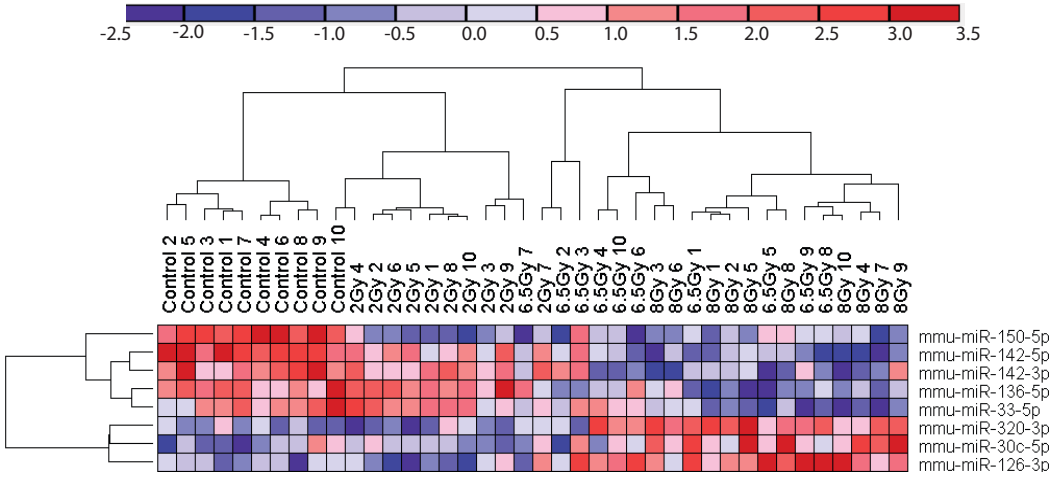


Fig.S6: MiRNAs significantly altered in all irradiated samples. Heatmap showing expression changes in levels of eight miRNAs significantly altered in all irradiated samples (2 Gy, 6.5 Gy, and 8 Gy) with respect to un-irradiated controls (0 Gy). Hierarchical clustering was performed to depict relationship between samples.

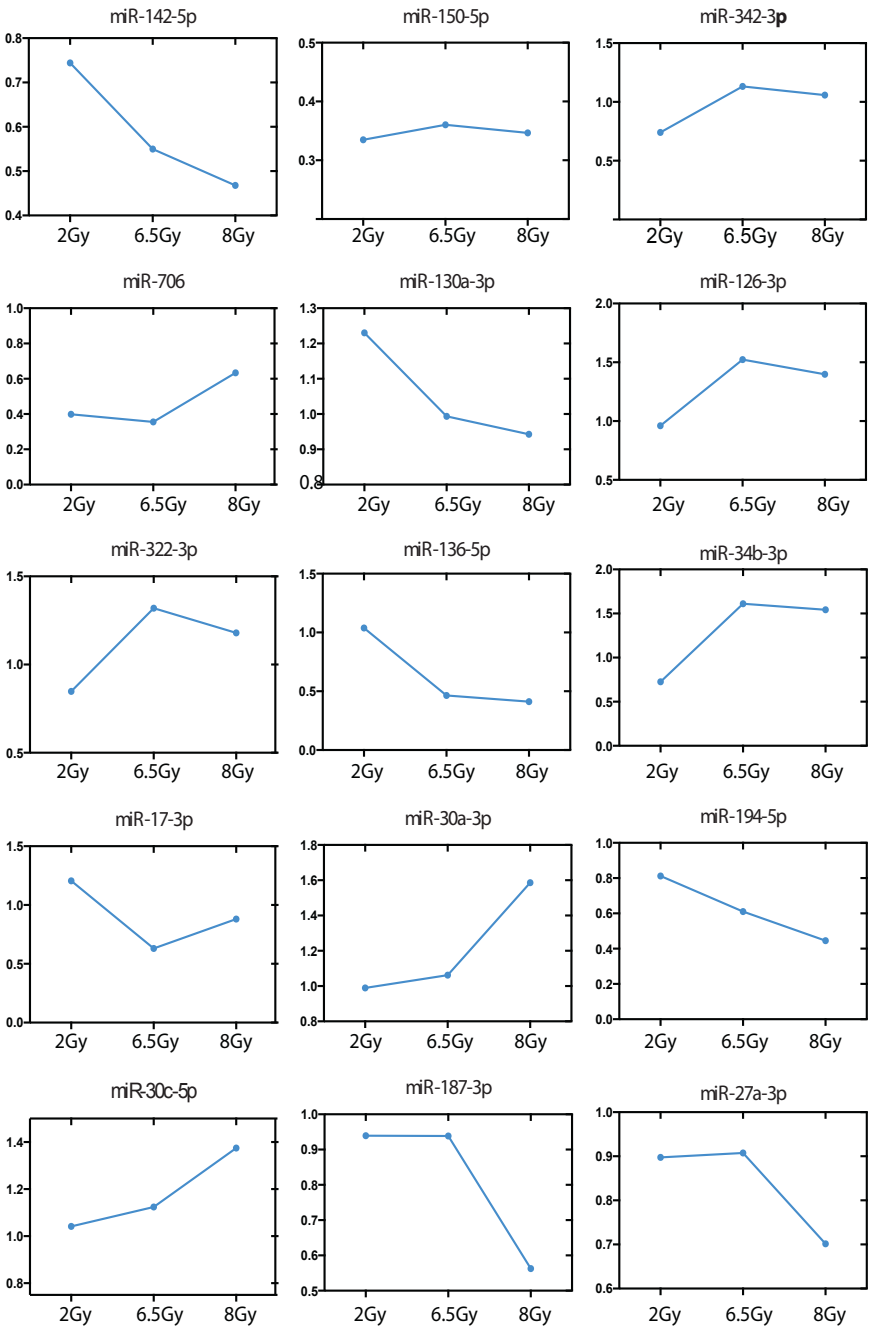


Fig.S7: Radiation dose-dependence of select miRNAs. In the original profiling experiment, C57BL6/J mice were exposed to three different doses of radiation, specifically 2, 6.5, and 8 Gy. Fold changes of select miRNAs in the sera of mice after exposure to different doses of radiation are presented. For each dose, fold change was calculated with respect to miRNA levels in control untreated samples.

A

miRNA	*P* value
miR-187-3p	0.0013
miR-194-5p	0.0016
miR-27a-3p	0.0063
miR-30a-3p	<0.0001
miR-30c-5p	0.0006

B

miRNA	CT + Saline	CT + Ami	TBI + Ami
miR-187-3p TBI + Saline	0.0009	0.0046	0.0034
miR-194-5p TBI + Saline	0.0022	0.0013	0.0097
miR-27a-3p TBI + Saline	0.0182	0.0183	0.0038
miR-30a-3p TBI + Saline	0.0002	<0.0001	0.0003
miR-30c-5p TBI + Saline	0.0023	0.0005	0.0018

Fig. S8: MiRNAs in the 6.5 v. 8 Gy signature as predictors of post-TBI survival. To study whether miRNAs in the 6.5 versus 8 Gy signature are predictors of animal viability, mice were treated with amifostine 1 hr prior to 8.5 Gy TBI dose. **(A)** Differences in expression values of indicated miRNAs in the four treatment groups (CT+ Saline, CT + Ami, 8.5 Gy + Saline, 8.5 Gy + Ami) were compared by one-way ANOVA and *P* values are presented. All five miRNAs showed significant differences. **(B)** *P* values for comparison of specific miRNA expression changes between the 8.5 Gy + Saline group and the other three experimental groups are presented. All miRNA levels were significantly altered in the 8.5 Gy + Saline group compared to the other three groups. Statistical significance assessed by one-way ANOVA followed by Dunnett's test.

Fig. S9: Identical mature miRNA sequences in human and mouse. Genomic sequences of miRNAs in the 6.5 vs. 8 Gy signature were aligned using microRNAviewer <http://people.csail.mit.edu/akiezun/microRNAviewer/index.html> (*47*). Mature sequences are highlighted in blue. Asterisks indicate base conservation.

Table S1: Peripheral blood CBC levels in individual C57BL/6J mice following TBI

Group	Mouse #	DAY 1					DAY 7					DAY 15					DAY 30				
		WBC (K/uL)	RBC (M/uL)	Hb (g/dL)	HCT (%)	PLT (K/uL)	WBC (K/uL)	RBC (M/uL)	Hb (g/dL)	HCT (%)	PLT (K/uL)	WBC (K/uL)	RBC (M/uL)	Hb (g/dL)	HCT (%)	PLT (K/uL)	WBC (K/uL)	RBC (M/uL)	Hb (g/dL)	HCT (%)	PLT (K/uL)
0 Gy	1	5.24	8.91	12.40	41.00	674	6.58	9.63	14.30	50.50	810	5.52	10.11	14.10	52.80	809	6.16	9.49	12.00	49.20	862
	2	8.36	9.66	14.00	45.70	883	7.30	10.47	14.20	54.50	787	7.70	10.33	13.90	52.50	899	3.84	10.20	12.90	52.50	852
	3	7.16	9.32	13.60	44.30	828	11.86	9.87	14.40	51.10	745	5.80	9.47	13.80	49.10	794	9.32	9.69	12.70	49.80	762
	4	6.88	9.28	14.00	47.80	900	6.64	9.99	14.10	52.60	771	5.70	9.63	13.30	49.30	820	4.98	9.52	12.00	49.50	927
	5	7.70	9.70	13.50	45.90	908	7.32	10.04	14.10	53.70	821	3.14	9.92	13.90	50.90	827	4.48	9.92	12.30	50.10	845
	Avg	7.07	9.37	13.50	44.94	838.60	7.94	10.00	14.22	52.48	786.80	5.57	9.89	13.80	50.92	829.80	5.76	9.76	12.38	50.22	849.60
	SEM	0.52	0.14	0.29	1.13	43.45	0.99	0.14	0.06	0.76	13.61	0.73	0.16	0.13	0.77	18.18	0.97	0.13	0.18	0.59	26.31
2 Gy	6	2.16	9.28	13.70	43.50	750	1.94	8.88	12.10	45.70	681	2.72	8.57	12.40	45.10	873	4.60	9.49	11.80	47.80	588
	7	1.92	9.60	13.60	45.20	928	2.20	9.11	12.90	47.40	662	2.74	9.03	12.70	47.00	698	2.64	9.39	12.60	48.40	667
	8	1.18	8.17	11.50	37.50	768	2.02	8.98	12.90	46.50	863	3.04	8.81	12.40	45.90	811	5.22	8.91	12.10	46.10	653
	9	2.18	9.27	13.10	42.90	793	2.18	9.41	12.80	48.90	684	3.04	9.47	13.00	49.10	707	4.26	8.84	11.80	44.90	143
	10	2.02	8.89	12.80	41.80	682	2.02	9.24	13.10	49.50	745	2.04	9.03	12.70	47.60	746	5.60	9.28	12.40	47.20	925
	Avg	1.89	9.04	12.94	42.18	784.20	2.07	9.12	12.76	47.60	727.00	2.72	8.98	12.64	46.94	767.00	4.46	9.18	12.14	46.88	595.20
	SEM	0.18	0.25	0.40	1.29	40.39	0.05	0.09	0.17	0.71	36.75	0.18	0.15	0.11	0.69	33.15	0.51	0.13	0.16	0.62	126.85
6.5 Gy	11	0.80	10.17	14.40	46.40	808	0.32	7.00	9.50	36.70	222	0.88	5.07	6.70	24.70	387	10.00	8.50	10.90	44.50	648
	12	1.12	9.93	14.10	45.70	855	0.54	7.62	10.60	39.10	205	1.18	5.92	8.50	30.30	381	9.06	8.32	11.30	44.80	536
	13	0.80	6.76	9.30	30.20	563	0.36	7.55	10.50	39.20	205	1.02	5.62	7.60	28.90	286	9.82	8.39	11.30	45.00	471
	14	1.14	9.48	13.20	43.60	964	0.30	7.43	10.10	38.00	183	0.56	5.58	7.50	27.60	357	9.12	8.25	11.20	43.80	723
	15	1.42	9.43	13.30	42.80	786	0.70	7.10	9.50	36.70	242	0.82	5.97	7.50	28.70	368	4.62	7.82	11.20	41.80	528
	Avg	1.06	9.15	12.86	41.74	795.20	0.44	7.34	10.04	37.94	211.40	0.89	5.63	7.56	28.04	355.80	8.52	8.26	11.18	43.98	581.20
	SEM	0.12	0.61	0.92	2.96	65.67	0.08	0.12	0.24	0.55	9.84	0.10	0.16	0.29	0.94	18.21	0.99	0.12	0.07	0.58	45.60
8 Gy	16	1.16	9.65	13.60	44.80	795	0.26	7.98	11.10	41.20	140	0.24	3.58	4.70	17.70	126					
	17	1.30	9.56	13.90	45.10	824	0.42	7.94	10.70	40.00	209	0.24	5.23	7.10	26.80	161					
	18	1.38	8.93	12.80	41.80	706	0.26	7.24	10.00	37.10	180	0.32	4.02	5.10	18.80	77					
	19	1.46	9.44	13.80	43.90	763	0.60	7.07	9.60	36.10	227	0.28	3.14	3.90	14.50	53					
	20	1.76	9.36	13.40	43.40	797	0.34	6.87	9.80	34.60	159	0.24	4.74	6.10	22.50	91					
	Avg	1.41	9.39	13.50	43.80	777.00	0.38	7.42	10.24	37.80	183.00	0.26	4.14	5.38	20.06	101.60					
	SEM	0.10	0.12	0.19	0.59	20.21	0.06	0.23	0.28	1.23	15.88	0.02	0.38	0.56	2.11	18.98					

Table S1: Peripheral blood CBC levels in individual mice. Complete Blood Cell (CBC) counts of C57BL/6J mice at 24 hours (day 1), and 7, 15, and 30 days post TBI. WBC, RBC, Hb, HCT, and PLT values for individual mice used in the experiment are listed. Five mice were used per TBI group per time point. Average and SEM are calculated per group. Abbreviations: WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; PLT, platelets; Mse#, mouse number; Avg, average; SEM, standard error of mean.

Table S2: Fold changes of 68 statistically significant miRNAs at different doses of TBI with respect to controls

miRNA	Rank	Benjamini-Hochberg corrected p-value	Fold change		
			2Gy vs 0Gy	6.5Gy vs 0 Gy	8Gy vs 0Gy
mmu-miR-142-5p	1	6.14552E-10	0.74424399	0.549934615	0.467752484
mmu-miR-150-5p	2	6.41729E-10	0.33485287	0.360400719	0.346659291
mmu-miR-320-3p	3	6.83112E-06	0.96224966	1.187536684	1.32071546
mmu-miR-136-5p	4	8.17096E-06	1.03771902	0.464873503	0.411761228
mmu-miR-33-5p	5	4.8463E-05	1.00605562	0.547886821	0.412110487
mmu-miR-142-3p	6	4.8463E-05	0.91027254	0.578995013	0.512969604
mmu-miR-30c-5p	7	0.000365421	1.04125706	1.124307627	1.374873802
mmu-miR-126-3p	8	0.000365421	0.96088497	1.52362955	1.397680627
mmu-miR-706	9	0.000466025	0.39885132	0.355518689	0.633605238
mmu-miR-375-3p	10	0.000466244	1.23386818	2.136008605	2.283729398
mmu-miR-29a-5p	11	0.000466244	0.88815734	0.965287846	0.709055154
mmu-miR-193a-3p	12	0.000529968	1.16807721	0.648857482	0.436360524
mmu-miR-99b-5p	13	0.000529968	0.99604663	1.343865649	1.512510076
mmu-miR-30a-3p	14	0.001068664	0.98962715	1.062153376	1.586158278
mmu-miR-194-5p	15	0.001068664	0.81191712	0.610441938	0.445697163
mmu-miR-151-3p	16	0.001068664	1.13039179	1.466557685	1.309094574
mmu-let-7d-3p	17	0.001068664	0.97075842	1.220688226	1.202449856
mmu-miR-486-5p	18	0.001406802	1.18781032	1.463315368	2.065511432
mmu-miR-423-5p	19	0.001406802	1.04859246	1.296138995	1.433861551
mmu-miR-30b-5p	20	0.002090685	1.03949322	1.118894183	1.226753205
mmu-miR-191-5p	21	0.002342556	1.13886399	1.431738514	1.633205493
mmu-miR-497-5p	22	0.003354593	0.93721128	0.749399546	0.620269054
mmu-miR-32-5p	23	0.003528045	1.08012807	0.628353232	0.60521677
mmu-miR-214-5p	24	0.003991952	0.7250106	0.703128367	0.472213492
mmu-miR-326-3p	25	0.005363873	1.23852539	0.851291436	0.795937744
mmu-miR-1195	26	0.00547774	0.96775855	1.091070893	1.828845971
mmu-miR-122-5p	27	0.00547774	0.9331905	0.347893687	0.148560227
mmu-miR-1839-3p	28	0.006649678	1.31726438	1.533746054	2.030902658
mmu-miR-500-3p	29	0.007061575	0.98538558	0.798727717	0.576675503
mmu-miR-30e-3p	30	0.00842863	0.99620407	1.118186905	1.447594383
mmu-miR-191-5p	31	0.008475716	1.11075355	1.336579832	1.549260059
mmu-miR-322-3p	32	0.008828838	0.84830107	1.319445753	1.179301198
mmu-miR-709	33	0.012398254	1.17074719	1.283596703	2.015808092
mmu-miR-486-3p	34	0.012398254	1.15572492	1.26343094	2.007373735
mmu-miR-133a-3p	35	0.01300781	0.8577409	1.808599164	2.388619602
mmu-miR-676-3p	36	0.013062937	1.02467973	1.200462931	1.261017633
mmu-miR-744-5p	37	0.013450652	1.11874039	1.206177008	1.300646391
mmu-miR-27a-3p	38	0.013750505	0.897455	0.907534042	0.701532751
mmu-miR-29a-3p	39	0.014568628	0.93057734	0.846667907	0.759548456
mmu-miR-1839-5p	40	0.014568628	1.08694504	1.276875982	1.316700195
mmu-miR-30a-5p	41	0.014568628	1.05482542	1.181824227	1.346126285
mmu-miR-199b-5p	42	0.016705178	0.86410859	0.513797016	0.632615767
mmu-miR-125a-5p	43	0.022628544	0.95885072	1.091894886	1.262232335
mmu-miR-133b-3p	44	0.024815118	0.87153511	1.658615981	2.115999503
mmu-miR-24-3p	45	0.024815118	0.98024783	1.184038592	1.119192515
mmu-miR-21a-5p	46	0.024815118	1.11453515	0.83129145	0.789893731
mmu-miR-503-5p	47	0.024815118	1.17062491	0.805712381	0.783530294
mmu-miR-328-3p	48	0.024815118	1.13325302	1.308132313	1.338479397
mmu-let-7g-5p	49	0.024815118	1.0988627	1.139369002	1.416967729
mmu-miR-362-3p	50	0.024815118	0.88814566	0.804011505	0.662542946
mmu-miR-199a-5p	51	0.025154963	0.90437047	0.601503597	0.631100613
mmu-miR-342-3p	52	0.02747761	0.74026498	1.131865806	1.05831998
mmu-miR-34b-3p	53	0.028987297	0.72634533	1.610402809	1.543163375
mmu-miR-15a-3p	54	0.028987297	1.1168344	0.719823542	0.588058284
mmu-miR-139-5p	55	0.033183048	0.89176006	1.179771672	1.070031762
mmu-miR-17-3p	56	0.033183048	1.20541903	0.631158748	0.880193062
mmu-miR-130a-3p	57	0.033183048	1.23030213	0.993397276	0.942905848
mmu-miR-149-5p	58	0.033183048	0.91083128	1.293986617	1.527284883
mmu-miR-29b-3p	59	0.033183048	1.02341004	0.816190497	0.738902258
mmu-miR-1a-3p	60	0.035135178	0.70860665	1.329943292	2.129491746
mmu-miR-23b-3p	61	0.036567207	0.96591806	1.179012129	1.113184704
mmu-miR-215-5p	62	0.036567207	0.74514845	0.695719558	0.474670243
mmu-miR-204-5p	63	0.040650135	0.85603509	1.631185521	1.807800285
mmu-miR-187-3p	64	0.041980065	0.93954282	0.938443632	0.562868937
mmu-miR-200b-5p	65	0.041980065	1.1873809	1.538043311	1.668073737
mmu-miR-25-3p	66	0.041980065	1.0929659	1.170620915	1.537301555
mmu-miR-338-3p	67	0.046950851	1.09509996	0.857254876	0.812354606
mmu-miR-196b-5p	68	0.049109597	1.31602496	1.065034064	0.733581783

Table S2: Fold change of serum miRNAs differentially expressed in mice exposed to different TBI doses at 24 hours. The table ranks miRNAs based on the Benjamini-Hochberg corrected p-value (see Materials and Methods). Of the 224 miRNAs analyzed for differential expression, 68 had a p value lower than 0.05, and are presented here.

Table S3: Percent human CD45 positive engraftment in individual huCD34+ NSG 'humanized' mice

Mouse #	% hCD45+ engraftment in mouse peripheral blood	Treatment
1	75.2	TBI + Saline
2	49.8	TBI + Saline
3	52.1	TBI + Saline
4	60.2	TBI + Saline
5	50.9	TBI + Saline
6	65	TBI + Saline
7	55.8	TBI + Saline
8	58.2	TBI + Amifostine
9	52.5	TBI + Amifostine
10	62.1	TBI + Amifostine
11	74.3	TBI + Amifostine
12	67.5	TBI + Amifostine
13	52.1	TBI + Amifostine
14	49.6	TBI + Amifostine
15	61.4	TBI + Amifostine
16	51.8	Control
17	50.8	Control
18	55.9	Control
19	58.8	Control
20	65.8	Control

Table S3: Percent human CD45+ cell engraftment in individual huCD34+ NSG mice. Vendor provided engraftment percentages analyzed at 12 weeks after transplantation. Animals were grouped into three groups, namely Control untreated (n =5), TBI + Saline (n = 7), and TBI+ Amifostine (n = 8). Human CD45+ cell engraftment

was reconfirmed at the end of the experiment in control untreated animals. Refer to Materials and Methods for details.

Table S4: Peripheral blood CBC levels in individual huCD34+ NSG 'humanized' mice

Group	Mouse #	CBC at euthanesia				
		WBC (K/uL)	RBC (M/uL)	Hb (g/dL)	HCT (%)	PLT (K/uL)
TBI + Saline	2	1.20	0.24	1.90	1.60	20
	5	1.30	0.19	2.80	1.20	4
	7	0.56	1.64	2.80	3.00	88
	Avg	1.02	0.69	2.50	1.93	37.33
	SEM	0.23	0.48	0.30	0.55	25.75
TBI + Amifostine	8	1.28	2.55	4.80	17.00	213
	9	1.19	1.65	3.20	11.00	44
	13	1.20	2.92	4.80	4.80	56
	Avg	1.22	2.37	4.27	10.93	104.33
	SEM	0.03	0.38	0.53	3.52	54.44
Control	16	1.20	4.75	11.00	38.50	345
	17	1.32	5.35	10.50	39.60	609
	18	2.16	5.66	12.40	42.20	686
	19	1.77	5.89	11.60	45.30	645
	20	1.44	5.41	10.70	40.60	577
	Avg	1.58	5.41	11.24	41.24	572.40
	SEM	0.17	0.19	0.34	1.18	59.69

Table S4: Peripheral blood CBC levels in individual huCD34+ NSG 'humanized' mice. Peripheral blood was harvested from moribund TBI treated animals or unirradiated controls. Average and SEM of CBC parameters are calculated per group. Abbreviations: WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; PLT, platelets; Avg, average; SEM, standard error of mean.

Table S5: Target sequences of individual miRNAs detected using Pick-N-Mix plates.

	mIRNA	Target Sequence
Control miRNA	mmu-miR-101a-3p	UACAGUACUGUGAUAACUGAA
Control miRNA	mmu-miR-19b-3p	UGUGCAAAUCCAUGCAAAACUGA
RNA Spike-in	UniSp6	Exiqon Proprietary Sequence
DNA Spike-in	UniSp3	Exiqon Proprietary Sequence
0 Gy v. 2 Gy Signature	mmu-miR-130a-3p	CAGUGCAAUGUUAAAAGGGCAU
	mmu-miR-142-5p	CAUAAAGUAGAAAGCACUACU
	mmu-miR-150-5p	UCUCCCAACCCUUGUACCAGUG
	mmu-miR-706	AGAGAAACCCUGUCUCAAAAAA
	mmu-miR-342-3p	UCUCACACAGAAAUCGCACCCGU
2Gy v. 6.5 Gy Signature	mmu-miR-34b-3p	AAUCACUAACUCCACUGCCAUC
	mmu-miR-322-3p	AAACAUGAAGCGCUGCAACAC
	mmu-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG
	mmu-miR-17-3p	ACUGCAGUGAGGGCACUUGUAG
	mmu-miR-136-5p	ACUCCAUUUGUUUUGAUGAUGG
6.5 Gy v. 8 Gy Signature	mmu-miR-187-3p	UCGUGUCUUGUGUUGCAGCCGG
	mmu-miR-194-5p	UGUAACAGCAACUCCAUGUGGA
	mmu-miR-27a-3p	UUCACAGUGGCUAAGUUCCGC
	mmu-miR-29a-3p	UAGCACCAUCUGAAAUCGGUUA
	mmu-miR-30a-3p	CUUUCAGUCGGAUGUUUGCAGC
	mmu-miR-30c-5p	UGUAAACAUCCUACACUCUCAGC

Table S5: Target sequences of individual miRNAs detected using Pick-N-Mix plates. MiRNAs that are part of the three signatures reported in Fig. 3 and their target sequences are shown. In addition, sequences for miR-101a and miR-19b are also listed. UniSp6 and UniSp3 sequences are proprietary (Exiqon).